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# Process and device for suspending heavy particles of a solid in a liquid

Insert A

The present invention relates to the suspending of particles of a solid in a predetermined volume of liquid. More particularly, the invention deals with the suspending, or re-suspending, of particles when they are contained in a container, with a predetermined volume of liquid, the particles being partially collected in the form of a deposit or sediment immersed by the liquid at the bottom of the container.

The particles/liquid physical state previously described and defined is encountered in particular in certain analysis protocols or processes, especially biological ones, involving relatively heavy particles, for example each consisting of a magnetic substrate to which, for example, a reagent or an analyte is bound. In order to implement or continue the analysis process, it is essential to suspend or re-suspend the particles in the predetermined volume of liquid inside the container, since failing this the particles which have sedimented are removed from the analysis process and vitiate its result in terms of reliability, sensitivity and reproducibility.

Until now, these particles have been suspended by mechanical or fluidic means, for example by passing a gas stream through the liquid volume in the container, close to or in contact with the deposit of sedimented particles. An operation of this type generally leads to the formation of foam, at the level of the interface between the liquid volume and the atmosphere internal to the container; it therefore has to be controlled carefully in order to limit, and if possible eliminate, the formation of foam which, in particular, hampers any subsequent optical measurement taken through the container. In all, this suspending or re-suspending of particles starting from a deposit at the bottom of a container, with everything in a

predetermined volume of liquid, represents an intricate and relatively time-consuming operation.

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The present invention therefore relates to a suspending process which is relatively "gentle" while remaining efficient, in so far as it does not significantly disturb the interface between the predetermined volume of liquid and the gas atmosphere contained in the container, for example an analysis cuvette.

According to the present invention, it has unexpectedly been discovered that the desired result can be obtained by setting up a gas circuit in the container, partly in direct contact with the liquid, in a loop comprising at least two substantially parallel flows which are separated by a head loss located level with the bottom of the container, and alternating a gas stream travelling through the said container along the said gas circuit.

Preferably, inert solid beads are arranged freely at the bottom of the container.

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~~The present invention is now described with reference to the appended single figure which schematically represents a vertical section of a device according to the invention.~~

¶ As shown in Fig. 1, the ~~A~~ device according to the invention generally comprises:

- a container 3 for holding a predetermined volume 2 of liquid, and particles 1 of a solid which are normally dispersed inside the container, in the volume of liquid, for example an aqueous phase; this container 3 has a flat bottom 3a and a neck 3b

- at least one conduit 10 which is arranged and penetrates inside the container 3, one end of which emerges from the neck 3b, forming a tubular gap with the latter, and the perforated other end 6 of which is located level with and against the bottom 3a of the container, thus forming, as described below, a head loss localized between the lower periphery of the conduit 3 and the bottom 3a opposite

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- a stopper 13 which closes off the upper end of the conduit 10 and in which an axial passage 13a is formed

- and solid inert beads 9 arranged freely on the bottom 3a of the container.

If necessary, the conduit 10/stopper 13 combination forms a component which is independent of the container 3 and can be introduced and extracted from the container 3, in order to suspend or re-suspend the particles 1 which will be discussed below.

The result of the structure or arrangement described above is that, in relation with the container 3, the conduit 10 defines two chambers 11 and 12, one which is external with respect to the conduit 10 and another which is internal to the conduit 10, communicating with one another through at least one gap or passage 6 which has been described above, level with the bottom 3a of the container 3, and which generates during operation the head loss which will be discussed below. These two chambers 11 and 12 communicate with the outside, respectively through the tubular gap 3, level with the neck 3b, and the opening 8 consisting of the axial channel 13a in the stopper 13. In this way, a gas circuit shown by the dot and dash line 5 can be set up in the container 3, passing through the opening 7, the chamber 11, the passage 6, the chamber 12 and the opening 8, or the reverse.

A means 14 of alternate pressurization is applied to the stopper 13, in relation with the opening 8, and makes it possible to set up a positive pressure then a negative pressure successively in the gas circuit 5 described above.

Irrespective of the relevant direction of the gas stream, the means 14 of alternate pressurization makes it possible for the circuit 5 shown by a dot and dash line in the single figure to be set up in the container 3, partly in direct contact with the liquid 2, in a loop or hair pin, comprising two substantially parallel flows 5a and 5b which circulate in the

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chambers 11 and 12 respectively and are separated by the head loss 6 located level with the bottom 3a of the container 3. Further, operation of the means 14 makes it possible to alternate the gas stream passing through the container 3 in the circuit 5 described above.

The gas circuit thus set up enters or leaves the container 3 through the two openings 7 and 8, each for inlet or outlet of the two flows 5a and 5b respectively, which are formed in the container 3 and are isolated from one another. During operation, the means 14 alternately applies a positive pressure then a negative pressure through the opening 8.

The following operating conditions or parameters may be considered:

- the head loss represents at least 10 mbar, and is preferably between 10 mbar and 500 mbar, and is for example between 50 mbar and 200 mbar
- the gas stream is alternated at a frequency at least equal to 3 Hz, and is preferably between 4 Hz and 25 Hz, for example between 5 Hz and 10 Hz.

**Example 1: Effect of the alternation frequency of the gas flow**

Estapor M1 070/60 particles coated with alkaline phosphatase and diluted in an estradiol buffer (Tris NaCl Prionex 5g/l) are re-suspended after one night of sedimentation at room temperature (concentration of the particles: 100µg/ml). The frequencies applied are respectively 2.5 Hz and 11 Hz. The percentage re-suspended was obtained by negative weighing according to the document FR-A-2 710 410 using a Mettler AE 240 magnetic balance modified to the requirements of the experiment. The error connected with the accuracy of the measurement is + or - 2%.

The results are presented in table 1 below and in the appended graph, according to Figure 2, in which:

- the ordinate represents the percentage re-suspended

- the abscissa represents the treatment time, expressed in seconds

- the black squares are assigned to the results obtained with a frequency of 2.5 Hz, and the white squares to the results obtained with a frequency of 11 Hz.

T60X

**Table 1**

| Re-suspension time in seconds. | Percentage re-suspended at a frequency of 2.5 Hz. | Percentage re-suspended at a frequency of 11 Hz. |
|---|---|---|
| 0 | 34.8 | 39 |
| 2 | 47.9 | 83.3 |
| 4 | 65.3 | 98.9 |
| 6 | 73.6 | 100* |
| 8 | 85.5 | 100* |
| 10 | 84.5 | 100* |

* indicates an error of + or - 2%.

As shown by the above table and the appended graph according to figure 2 for a frequency of 11 Hz, the frequency plays an important role in the process of the invention. 100% re-suspension is obtained after an agitation time of at least 6 seconds at a frequency of 11 Hz, while it is impossible to obtain a homogeneous suspension, even after 10 seconds of agitation, at a frequency of 2.5 Hz.

**Example 2: Effect of the beads on the re-suspension**

Seradyn C942339 particles coated with alkaline phosphatase and diluted in an estradiol buffer (Tris NaCl Prionex 5g/l) were re-suspended after one month of sedimentation at a temperature of between 2 and 8°C (concentration of the particles: 100µ/ml). The frequency applied is 11 Hz. The percentage re-suspended was obtained by negative weighing according to patent FR-A-2 710 410 using a Mettler AE 240 magnetic balance modified to the requirements of the experiment. The

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error connected with the accuracy of the measurement is + or - 2%.

The results are presented in Table 2 below and in the appended graph according to Figure 3, in which:

- the abscissa and ordinate express the same quantities and scales as those represented in Fig. 2

- the black squares are assigned to the results without beads, and the white squares to the results with 5 mm glass beads.

T70X

**Table 2**

| Re-suspension time in seconds. | Percentage re-suspended without any beads. | Percentage re-suspended with 5mm glass beads. |
|---|---|---|
| 0 | 0 | 0 |
| 2 | 81 | 100* |
| 4 | 93 | 100* |
| 6 | 100* | 100* |
| 8 | 100* | 100* |
| 10 | 100* | 100* |

* indicates an error of + or - 2%.

As shown by the above table and the appended graph according to Fig. 3, the addition of glass beads plays an essential role in the process of the invention. 100% re-suspension is obtained after an agitation time of at least 2 seconds at a frequency of 11 Hz in the presence of glass beads, while in the absence of glass beads it is possible to obtain a homogeneous suspension only after 6 seconds of agitation.

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